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(FILE 'HOME' ENTERED AT 12:33:10 ON 04 AUG 2003)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 12:33:19 ON 04 AUG 2003

SEA CELLULASE OR ENDOGLUCANASE

3	FILE ADISCTI
1	FILE ADISNEWS
3607	FILE AGRICOLA
143	FILE ANABSTR
302	FILE AQUASCI
1849	FILE BIOBUSINESS
169	FILE BIOCOMMERCE
9755	FILE BIOSIS
6409	FILE BIOTECHABS
6409	FILE BIOTECHDS
3071	FILE BIOTECHNO
5632	FILE CABA
40	FILE CANCERLIT
17737	FILE CAPLUS
2089	FILE CEABA-VTB
24	FILE CEN
111	FILE CIN
274	FILE CONFSCI
168	FILE CROPB
227	FILE CROPU
76	FILE DDFB
37	FILE DDFU
4741	FILE DGENE
76	FILE DRUGB
75	FILE DRUGLAUNCH
285	FILE DRUGMONOG2
51	FILE DRUGU
15	FILE EMBAL
3354	FILE EMBASE
2040	FILE ESBIOBASE
169	FILE FEDRIP
65	FILE FOREGE
880	FILE FROSTI
2351	FILE FSTA
2100	FILE GENBANK
21	FILE HEALSAFE
1372	FILE IFIPAT
1773	FILE JICST-EPLUS
5	FILE KOSMET
3741	FILE LIFESCI
3	FILE MEDICONF
2975	FILE MEDLINE
12	FILE NIOSHTIC
358	FILE NTIS
106	FILE OCEAN
5261	FILE PASCAL
11	FILE PHIN
353	FILE PROMT
20	FILE RDISCLOSURE
7151	FILE SCISEARCH
1	FILE SYNTHLINE
1828	FILE TOXCENTER

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            5195   FILE USPATFULL
             167   FILE USPAT2
              10   FILE VETB
             222   FILE VETU
            2729   FILE WPIDS
            2729   FILE WPINDEX
L1              QUE CELLULASE OR ENDOGLUCANASE
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     FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, CABA, PASCAL, LIFESCI,
     AGRICOLA, EMBASE, BIOTECHNO, MEDLINE, WPIDS, FSTA' ENTERED AT 12:34:34 ON
     04 AUG 2003
L2          231 S L1 AND ACIDOTHERMUS
L3          223 S L2 AND CELLULOLYTICUS
L4           39 S L3 AND (CELLULOSE BINDING DOMAIN OR CBD OR TYPE III OR TYPE
L5           19 DUP REM L4 (20 DUPLICATES REMOVED)
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L5 ANSWER 11 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:144350 BIOSIS
DOCUMENT NUMBER: PREV199900144350
TITLE: Molecular diversity of thermophilic cellulolytic and hemicellulolytic bacteria.
AUTHOR(S): Bergquist, Peter L. (1); Gibbs, Moreland D.; Morris, Daniel D.; Te'o, V. S. Junior; Saul, David J.; Morgan, Hugh W.
CORPORATE SOURCE: (1) Sch. Biol. Sci., Macquarie Univ., Sydney, NSW 2109 Australia
SOURCE: FEMS Microbiology Ecology, (Feb., 1999) Vol. 28, No. 2, pp. 99-110.
ISSN: 0168-6496.
DOCUMENT TYPE: General Review
LANGUAGE: English
AB Many thermophilic bacteria belong to groups with deep phylogenetic lineages and ancestral forms were established before the occurrence of eucaryotes that produced cellulose and hemicellulose. Thus they may have acquired their beta-glycanase genes from more recent mesophilic bacteria. Most research has focussed on extremely thermophilic eubacteria growing above 65degreeC under anaerobic conditions. Only recently have aerobic cellulolytic thermophiles been described from widely separated lineages (for example, Rhodothermus marinus, Caldibacillus cellulovorans). Many thermophilic bacteria produce **cellulases** and xylanases that have novel structures, with additional protein domains not identified with their catalytic activity. Many of these enzymes are multifunctional and code for more than one catalytic activity. This type of enzyme structure was first identified in the extreme thermophile Caldicellulosiruptor saccharolyticus. There is a general relatedness evident between catalytic domains, **cellulose binding domains** and other ancillary domains, which suggests that there may have been significant lateral gene transfer in the evolution of these microorganisms. Detailed molecular studies show that there is variation in the sequences of these related but not identical genes from taxonomically widely-separated organisms.

L5 ANSWER 10 OF 19 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-087663 [08] WPIDS
DOC. NO. CPI: C2000-024514
TITLE: Isolated domains of **Acidothermus cellulolyticus** E1 **endoglucanase** useful for labeling or modifying a cellulose and for purifying or immobilizing a binding domain fusion protein to cellulose.
DERWENT CLASS: C06 D16
INVENTOR(S): ADNEY, W S; HIMMEL, M E; LAYMON, R A; THOMAS, S R
PATENT ASSIGNEE(S): (MIDE) MIDWEST RES INST
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2226898	A1	19990925	(200008)*	EN	85

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
CA 2226898	A1	CA 1998-2226898	19980325

PRIORITY APPLN. INFO: CA 1998-2226898 19980325
AB CA 2226898 A UPAB: 20000215
NOVELTY - A compound comprising amino acid sequence (I) or a domain variant, consisting of residues 420-521 of the **Acidothermus cellulolyticus** E1 **endoglucanase** sequence (**II**) of 562 amino acids given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated and purified nucleic acid (**III**) encoding (I) or its domain variant;

(2) a hybrid protein (IV) comprising (I) or its domain variant;

(3) a nucleic acid encoding (IV);

(4) a chemical derivative (V) of (I);

(5) a chemical derivative (VI) of (IV);

(6) a method of modifying a polysaccharide surface comprising contacting the polysaccharide with (I) or a hybrid protein comprising (I);

(7) a method of purifying (IV) comprising contacting the fusion protein with a polysaccharide matrix;

(8) a method of immobilizing (IV) on a support comprising contacting the hybrid protein with a polysaccharide matrix;

(9) a method of producing (I), domain variant of (I) or a hybrid protein comprising (I) comprising:

(a) inserting a nucleic acid sequence encoding (I), domain variant or hybrid protein into a vector;

(b) introducing the vector into a host cell; and

(c) expressing the nucleic acid of step (a); and

(10) a domain variant of (**III**) encoding a compound which comprises a defined sequence of 110 amino acids given in the specification.

USE - The **cellulose binding domain** of the E1 **endoglucanase** (E1 **CBD**) is useful in labeling or modifying a cellulose or other polysaccharide surface and in purifying or immobilizing a binding domain fusion protein to cellulose or other polysaccharide. The modified cellulose can then be used in textile, pulp, paper, chemical and pharmaceutical industries. A fusion protein comprising E1 **CBD** can be used to modify the chemical or physical properties of a cellulose or polysaccharide matrix column and to roughen or disrupt a cellulose or polysaccharide fiber.

E1 **endoglucanase** is a beta -1,4-**endoglucanase** or endocellulase

ADVANTAGE - The **cellulose binding domain** of the E1 **endoglucanase** (E1 **CBD**) exhibits greater stability at pH 4-8 and has an optimum temperature for stability of 83 deg. C which is not found in **CBD** from organisms not adapted to thermal temperatures.

Dwg.0/7

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L5 ANSWER 1 OF 19 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-11180 BIOTECHDS
TITLE: Novel thermal tolerant GuxA polypeptide derived from **Acidothermus cellulolyticus**, useful for reducing cellulose in a starting material, and for the conversion of biomass to biofuels and biofuel additives; vector-mediated recombinant protein gene transfer and expression in host cell for use in carbohydrate degradation, cellulose degradation, fuel, surfactant, paper mill and monoclonal antibody preparation
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E; DECKER S R
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 2003012109 13 Feb 2003
APPLICATION INFO: WO 2001-US23817 28 Jul 2001
PRIORITY INFO: WO 2001-23817 28 Jul 2001; WO 2001-23817 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-239526 [23]
AB DERWENT ABSTRACT:

NOVELTY - An isolated thermal tolerant GuxA polypeptide (I) derived from **Acidothermus cellulolyticus**, having a sequence (S1) of 1228 amino acids (aa). Fragments of (I) e.g., first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (**CBD**) **type III**, and **CBD type II**, having 423, 231, 150, and 101 aas, respectively, is new. All sequences are fully disclosed in the specification.

DETAILED DESCRIPTION - An isolated thermal tolerant GuxA polypeptide (I) derived from **Acidothermus cellulolyticus**, having a sequence (S1) of 1228 amino acids (aa). Fragments of (I) e.g., first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (**CBD**) **type III**, and **CBD type II**, having 423, 231, 150, and 101 aas, respectively. (I) has a sequence (S1) of 1228 aas. The fragments of (I) such as first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (**CBD**) **type III**, and **CBD type II**, having 423, 231, 150, and 101 aas, respectively; or a sequence having at least 70% identity with the above sequences. INDEPENDENT CLAIMS are also included for the following; (1) composition (**II**) comprising (I); (2) an industrial mixture suitable for degrading cellulose, comprising (I); (3) fusion protein (**III**) comprising (I) and a heterologous peptide; (4) **cellulase**-substrate complex comprising (I) bound to cellulose; (5) vector comprising the polynucleotide that encodes (I); (6) host cell genetically engineered to express (I); (7) composition comprising (I) and a carrier; (8) isolated antibody (IV) that specifically binds to (I); (9) production of (I); (10) set of amplification primers (V) for amplification of a polynucleotide molecule encoding GuxA, comprising two or more sequences having 9 or more contiguous nucleic acids derived from the polynucleotide molecule; and (11) probe (VI) for hybridizing to a polynucleotide encoding GuxA comprising a sequence of 9 or more contiguous nucleic acid derived from the polynucleotide molecule.

WIDER DISCLOSURE - Also disclosed as new are the following: (1) recombinant forms of (I); (2) variants, and derivatives of (I); (3) reagents, compositions, and methods that are useful for analysis of GuxA activity; and (4) a polynucleotide encoding (I).

BIOTECHNOLOGY - Preparation: (I) is produced by incubating the above mentioned host cell (claimed). Preferred Mixture: The industrial mixture further comprises a detergent. Preferred Fusion Protein: The heterologous peptide is a leucine zipper.

USE - The set of primers (V) are useful for the detection of a

polynucleotide encoding GuxA, by amplifying a nucleic acid sequence with (V), and correlating the amplified nucleic acid sequence with detected polynucleotide encoding GuxA. (I) is useful for assessing the carbohydrate degradation activity of Gux A, by analyzing a carbohydrate degradation in the presence of GuxA and a carbohydrate degradation in the absence of GuxA on a substrate, and comparing the carbohydrate degradation in the presence of GuxA with the carbohydrate degradation in the absence of GuxA. The method is also carried out in the presence of desired agent. (I) is useful for reducing cellulose in a starting material such as agricultural biomass (all claimed), to sugars which is useful in biofuel production. (I) is useful in the conversion of biomass to biofuels and biofuel additives, in detergents pulp and paper processing, food and feed processing, and in textile process. Fragments of (I) are useful to generate specific anti-GuxA antibodies. (I) is also useful to raise polyclonal and monoclonal antibodies that are useful in purifying GuxA, or detecting GuxA polypeptide expression, and as well as reagent tool for characterizing the molecular actions of GuxA polypeptides.

EXAMPLE - Molecular cloning of GuxA was as follows: Genomic DNA was isolated from **Acidothermus cellulolyticus** and purified. The purified genomic DNA was then digested and separated on agarose gels. DNA fragments in the range of 9-20 kilobase pairs were isolated from the gels. This purified genomic DNA was ligated into the BamH1 acceptor site of purified EMBL3 lambda phage arms. Phage DNA was packaged and plated with Escherichia coli LE392 in top agar which contained the soluble cellulose analog, and carboxymethylcellulose. The plates were then incubated overnight to allow transfection, bacterial growth, and plaque formation. Plates were stained followed by destaining. lambda plaques harboring **endoglucanase** clones showed up as unstained plaques. lambda clones which screened positive were purified. Individual phage isolates were named SL-1, SL-2, SL-3, and SL-4. Subsequent subcloning efforts employed the SL-3 clone which contained 14.2 kb fragment of A.**cellulolyticus** genomic DNA. Template DNA was constructed using a 9 kb BamH1 fragment obtained from 14.2 kb lambda clone SL3 prepared from A.**cellulolyticus** genomic DNA. The 9-kb BamHI fragment from SL3 was subcloned into pDR540 to generate a plasmid NREL501. NREL501 was first sequenced, then subcloned into pUC19, and transformed into E.coli XL1-blue for the production of template DNA for sequencing. Each subclone was sequenced, and the sequencing data from primer walking and subclones were assembled together to verify that all SL3 regions had been sequenced. An open reading frames (ORF) was found in the 9-kb BamHI fragment, C-terminal of E1, and then termed as GuxA. An ORF of 3687 bp defined in the specification and its deduced aa sequences having 1228 aas were obtained. The aa sequence predicted was determined to have significant homology to known **cellulases**. (47 pages)

L5 ANSWER 2 OF 19 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-11423 BIOTECHDS
TITLE: New thermostable AviIII peptide from **Acidothermus cellulolyticus**, useful for degradation of cellulose or in generating anti-AviIII antibodies for purifying recombinant AviIII polypeptides from genetically engineered host cells;
recombinant Avicelase protein production useful for polysaccharide degradation
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 2003012090 13 Feb 2003
APPLICATION INFO: WO 2001-US23818 28 Jul 2001
PRIORITY INFO: WO 2001-23818 28 Jul 2001; WO 2001-23818 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-248177 [24]
AB DERWENT ABSTRACT:

NOVELTY - A thermostable AviIII peptide comprising a sequence of 1000 amino acids given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) A composition comprising a substantially purified thermostable AviIII peptide cited above which has a catalytic domain GH74 and a carbohydrate binding domain (**CBD**) **III**, and a carrier; (2) An industrial mixture suitable for degrading cellulose, comprising the thermostable AviIII polypeptide cited above; (3) An isolated polynucleotide molecule encoding the above thermostable AviIII polypeptide; (4) A fusion protein comprising the polypeptide cited above; (5) A **cellulase**-substrate complex comprising the above polypeptide bound to cellulose; (6) A vector comprising the above polynucleotide molecule; (7) A host cell genetically engineered to express the polynucleotide molecule cited above; (8) An isolated antibody that specifically binds to the above polypeptide; (9) Producing AviIII polypeptide, comprising incubating the host cell cited above; (10) A set of amplification primers for amplification of a polynucleotide molecule encoding the thermostable AviIII polypeptide, comprising 2 or more sequences having 9 or more contiguous nucleic acids derived from the polynucleotide molecule cited above; (11) A probe for hybridizing to the polynucleotide encoding AviIII, comprising a sequence of 9 or more contiguous nucleic acids derived from the polynucleotide molecule cited above; (12) Detecting a polynucleotide encoding the thermostable AviIII polypeptide, comprising amplifying a nucleic acid sequence with a set of amplification primers cited above, and correlating the amplified nucleic acid sequence with detected polynucleotide encoding a thermostable AviIII; and (13) Reducing cellulose in a starting material, comprising administering to the starting material an amount of the polypeptide cited above.

BIOTECHNOLOGY - Preferred Polypeptide: The thermostable AviIII polypeptide variants or derivatives may comprise S1 or a sequence of 740 (S2), 154 (S3) or 88 (S4) amino acids given in the specification, or a sequence that is about 70% identical to S1-4. Preferred Mixture: The industrial mixture further comprises a detergent. Preferred Polynucleotide: The polynucleotide comprises a sequence that is about 90% identical to a sequence of 3000 bp (S5) given in the specification, or a sequence that is about 90% identical to the nucleic acid sequence encoding S1. Preparation: The thermostable AviIII peptide was isolated from **Acidothermus cellulolyticus**

USE - The thermostable AviIII peptide is useful in the degradation of cellulose (claimed), and in generating specific anti-AviIII antibodies that are useful in purifying recombinant AviIII polypeptides from genetically engineered host cells, in detecting AviIII polypeptide expression, as well as a reagent tool for characterizing the molecular actions of the polypeptide. The polypeptide is also used in adding in delivery or targeting of other pharmaceutical compositions within a host. The polynucleotide is useful as probes or primers in various diagnostic assays. The mixture is useful in crude fermentation processing or other industrial processing.

ADMINISTRATION - Administration is by oral, parenteral (e.g. subcutaneous, intravenous, intramuscular, intrasternal or infusion techniques), by inhalation spray, topical, mucosal, or rectal means. No dosage given.

EXAMPLE - No relevant example given. (22 pages)

L5 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2003:435217 CAPLUS

DOCUMENT NUMBER: 139:19027

TITLE: Protein and DNA sequences of thermal tolerant **cellulase** GuxA from **Acidothermus cellulolyticus** and used in degrading cellulose in agricultural biomass and municipal solid waste

INVENTOR(S): Ding, Shi-You; Adney, William S.; Vinzant, Todd B.; Himmel, Michael E.; Decker, Stephen R.

PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003104522	A1	20030605	US 2001-917383	20010728

PRIORITY APPLN. INFO.: US 2001-917383 20010728

AB The invention provides protein and DNA sequences of a thermal tolerant **cellulase** GuxA that is a member of the glycoside hydrolase family. GuxA has been isolated and characterized from **Acidothermus cellulolyticus**. The invention further provides recombinant forms of the identified GuxA. Methods of making and using GuxA polypeptides, including fusions, variants, and derivs., are also disclosed. The invention further relates to the use of GuxA in making detergents and degrading cellulose in agricultural biomass and municipal sold waste.

L5 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:396366 CAPLUS
DOCUMENT NUMBER: 138:397248
TITLE: Thermal tolerant exoglucanase from **Acidothermus cellulolyticus**
INVENTOR(S): Adney, William S.; Ding, Shi-You; Vinzant, Todd B.; Himmel, Michael E.; Decker, Stephen R.; McCarter, Suzanne Lantz
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003096342	A1	20030522	US 2001-917384	20010728

PRIORITY APPLN. INFO.: US 2001-917384 20010728

AB The invention provides a thermal tolerant **cellulase** that is a member of the glycoside hydrolase family. The invention further discloses this **cellulase** as Gux1. Gux1 has been isolated and characterized from **Acidothermus cellulolyticus**.The invention further provides recombinant forms of the identified Gux1. Methods of making and using Gux1 polypeptides, including fusions, variants, and derivs., are also disclosed.

L5 ANSWER 5 OF 19 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-11426 BIOTECHDS
TITLE: Novel thermal tolerant mannanase A polypeptide derived from **Acidothermus cellulolyticus**, useful for reducing hemicellulose in a starting material, for processing of food, and as bulking agents in food stuffs; vector-mediated gene transfer and expression in host cell for recombinant enzyme production
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 2003012110 13 Feb 2003
APPLICATION INFO: WO 2001-US23819 28 Jul 2001
PRIORITY INFO: WO 2001-23819 28 Jul 2001; WO 2001-23819 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English

OTHER SOURCE: WPI: 2003-248182 [24]

AB DERWENT ABSTRACT:

NOVELTY - An isolated thermal tolerant mannanase A polypeptide (I) derived from **Acidothermus cellulolyticus**, comprising a sequence (S1) of 762 amino acids, and the fragments of (I) such as catalytic domain glycoside hydrolase (GH) 5, carbohydrate binding domain (**CBD**) **type III**, and **CBD type II**, having 411, 608, and 762 amino acids, respectively, is new. All sequences are given in the specification.

DETAILED DESCRIPTION - An isolated thermal tolerant mannanase A polypeptide (I) derived from **Acidothermus cellulolyticus**, comprising a sequence of 762 amino acids. The fragments of (I) such as catalytic domain, carbohydrate binding domain **type III**, and carbohydrate binding domain **type II**, have 411, 608, and 762 amino acids, respectively; or a sequence having at least 70% identity with the above sequences. All sequences are defined in the specification. INDEPENDENT CLAIMS are also included for: (1) a composition (**II**) comprising (I); (2) an industrial mixture suitable for degrading hemicellulose, comprising (I); (3) an isolated polynucleotide molecule (**III**) comprising a nucleic acid sequence having 90% sequence identity to a sequence encoding S1, or encoding a heterologous protein in frame with S1; (4) a mannanase substrate complex comprising (I) bound to hemicellulose; (5) a vector comprising (**III**); (6) a host cell genetically engineered to express (**III**); (7) a composition comprising (I) and a carrier; (8) an isolated antibody (IV) that specifically binds to (I); (9) production of (I); (10) a set of amplification primers (V) for amplification of a polynucleotide molecule encoding mannanase A, comprising two or more sequences having 9 or more contiguous nucleic acids derived from (**III**); and(11) a probe (VI) for hybridizing to a polynucleotide encoding mannanase A comprising a sequence of 9 or more contiguous nucleic acid derived from (**III**).

WIDER DISCLOSURE - Also disclosed are: (1) recombinant forms of (I); (2) variants, derivatives and fusion proteins of (I); and (3) reagents, compositions, and methods that are useful for analysis of ManA activity.

BIOTECHNOLOGY - Preparation: (I) is produced by incubating the above mentioned host cell (claimed). Preferred Mixture: The industrial mixture further comprises a detergent.

USE - (V) is useful for the detection of a mannanase A polynucleotide, by amplifying a nucleic acid sequence with (V), and correlating the amplified nucleic acid sequence with detected polynucleotide encoding mannanase A. (I) is useful for assessing the carbohydrate degradation activity of mannanase A, by analyzing a carbohydrate degradation in the presence of mannanase A and a carbohydrate degradation in the absence of mannanase A on a substrate, and comparing the carbohydrate degradation in the presence of mannanase A with the carbohydrate degradation in the absence of mannanase A. The method is also carried out in the presence of a desired agent. (I) is also useful for reducing hemicellulose in a starting material (claimed), to simpler carbohydrate units, ultimately to sugars which are useful in the food, feed, paper pulp, and biofuels industries. (I) is useful for the processing of food and in food stuffs as bulking agents. Fragments of (I) are useful to generate specific anti-ManA antibodies. (I) is also useful to raise polyclonal and monoclonal antibodies that are useful in purifying ManA, or detecting ManA polypeptide expression, and as well as reagent tool for characterizing the molecular actions of ManA polypeptides. (**II**) is useful for removal of hemicellulose containing stains within fabrics and in pulp and paper industry to address conditions associated with hemicellulose contamination of the cellulose fraction. (**II**) is also useful to produce oligosaccharide bulking agents and stabilizers from hemicellulose for use in the food and feed industry.

EXAMPLE - Molecular cloning of mannanase A (ManA) was as follows. Genomic DNA was isolated from **Acidothermus**

cellulolyticus and purified. The purified genomic DNA was then digested and separated on agarose gels. DNA fragments in the range of 9-20 kilobase pairs were isolated from the gels. This purified genomic DNA was ligated into the BamH1 acceptor site of purified EMBL3 lambda phage arms. Phage DNA was packaged and plated with Escherichia coli LE392 in top agar which contained the soluble cellulose analog, and carboxymethylcellulose. The plates were then incubated overnight to allow transfection, bacterial growth, and plaque formation. Plates were stained followed by destaining. lambda plaques harboring **endoglucanase** clones showed up as unstained plaques. lambda clones which screened positive were purified. Individual phage isolates were named SL-1, SL-2, SL-3, and SL-4. Subsequent subcloning efforts employed the SL-3 clone which contained 14.2 kb fragment of A. **cellulolyticus** genomic DNA. Template DNA was constructed using a 9 kb BamH1 fragment obtained from 14.2 kb lambda clone SL3 prepared from A. **cellulolyticus** genomic DNA. The 9-kb BamHI fragment from SL3 was subcloned into pDR540 to generate a plasmid NREL501. NREL501 was first sequenced, then subcloned into pUC19, and transformed into E. coli XL1-blue for the production of template DNA for sequencing. Each subclone was sequenced, and the sequencing data from primer walking and subclones were assembled together to verify that all SL3 regions had been sequenced. An open reading frame (ORF) was found in the 9-kb BamHI fragment, C-terminal of E1, and then termed as ManA. An ORF of 2289 bp defined in the specification and its deduced amino acid sequences having 762 amino acids were obtained. The amino acid sequence predicted was determined to have significant homology to known mannanases.(46 pages)

L5 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2003:114969 CAPLUS
DOCUMENT NUMBER: 139:32386
TITLE: Expression and import of an active **cellulase** from a thermophilic bacterium into the chloroplast both in vitro and in vivo
AUTHOR(S): Jin, Rongguan; Richter, Stefan; Zhong, Rong; Lamppa, Gayle K.
CORPORATE SOURCE: Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, 60637, USA
SOURCE: Plant Molecular Biology (2003), 51(4), 493-507
CODEN: PMBIDB; ISSN: 0167-4412
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A bacterial thermostable **cellulase**, the endo-1,4-.beta.-D-glucanase E1 from **Acidothermus cellulolyticus**, was imported into chloroplasts, and an active enzyme was recovered both in vitro and in vivo. Precursor fusion proteins were synthesized with E1 or its catalytic domain, CD, fused to the transit peptide of ferredoxin or ribulose-bisphosphate carboxylase activase for stromal targeting. A spacer region of 1, 5 or 15 amino acids was included carboxy to the transit peptide. The efficiency of import and processing by the stromal processing peptidase depended on the nature of the transit peptide and the passenger protein, and increased with the length of the spacer between them. Besides finding E1 or CD in the stroma, protein was arrested in the envelope during import showing that structural features of E1 and CD, along with their proximity to the transit peptide, influence translocation. The **cellulose binding domain** and/or serine/proline/threoline-rich linker of E1 may impede efficient import. Significantly, most precursors for E1 and CD synthesized by in vitro translation possessed endoglucanse activity that was temp.-dependent, and required the residues AGGGY at the N-terminus of E1 and CD. Furthermore, activity was detected upon import into chloroplasts. Based on the in vitro analyses, five precursor fusion proteins were selected to det. if E1 and CD would be successfully targeted to chloroplasts in vivo. In transgenic tobacco plants, E1 and CD accumulated

in both the stromal and membrane fractions and, importantly, chloroplast exts. showed **endoglucanase** activity.
REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 19 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2002:307549 SCISEARCH
THE GENUINE ARTICLE: 538AR
TITLE: 4-methyl-7-thioumbelliferyl-beta-D-cellobioside: A fluorescent, nonhydrolyzable substrate analogue for **cellulases**
AUTHOR: Barr B K (Reprint); Holewinski R J
CORPORATE SOURCE: Loyola Coll Maryland, Dept Chem, Baltimore, MD 21210 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (2 APR 2002) Vol. 41, No. 13, pp. 4447-4452. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The kinetics of cellulose binding and hydrolysis by **cellulases** is not well understood except at steady-state conditions. For use in studies of **cellulase** pre-steady-state and steady-state kinetics, we have prepared 4-methyl-7-thioumbelliferyl-beta-D-cellobioside (MUS-CB), a ground-state nonhydrolyzable analogue of the fluorescent **cellulase** substrate 4-methylumbelliferyl-beta-D-cellobioside (MU-CB). MUS-CB is not hydrolyzed by the catalytic domain of **cellulase** E1 from **Acidothermus cellulolyticus** under conditions where this enzyme rapidly degrades MU-CB. Thermodynamic parameters describing the steady-state binding of MUS-CB to Thermobifida fusca **cellulase** Cel6A are similar to those for MU-CB, indicating that MUS-CB can be used in place of MU-CB to study binding events in the Cel6A active-site cleft. In the pre-steady-state, MUS-CB binds to Cel6A by a simple, one-step bimolecular association reaction. It is anticipated that similar thio-containing 4-methylumbelliferyl compounds will have applications in studies of other enzyme systems.

L5 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2002:323625 CAPLUS
DOCUMENT NUMBER: 137:43352
TITLE: Exploration of cellulose surface-binding properties of **Acidothermus cellulolyticus** Cel5A by site-specific mutagenesis
AUTHOR(S): McCarter, Suzanne L.; Adney, William S.; Vinzant, Todd B.; Jennings, Edward; Eddy, Fannie Posey; Decker, Stephen R.; Baker, John O.; Sakon, Joshua; Himmel, Michael E.
CORPORATE SOURCE: Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA
SOURCE: Applied Biochemistry and Biotechnology (2002), 98-100(Biotechnology for Fuels and Chemicals), 273-287 CODEN: ABIBDL; ISSN: 0273-2289
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Understanding the interactions between **cellulases** and cellulosic substrates is crit. to the development of an efficient artificial **cellulase** system for conversion of biomass to sugars. We directed specific mutations to the interactive surface of the **Acidothermus cellulolyticus** E1 **endoglucanase** catalytic domain. The

cellulose-binding domain is not translated in these mutants. Amino acid mutations were designed either to change the surface charge of the protein or to modify the potential for hydrogen bonding with cellulose. The relationship between **cellulase** -to-cellulose (Avicel PH101) binding and hydrolysis activity was detd. for various groupings of mutations. While a significant increase in hydrolysis activity was not obsd., certain clusters of residues did significantly alter substrate binding and some interesting correlations emerged. In the future, these observations may be used to aid the design of **endoglucanases** with improved performance on pretreated biomass.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 19 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-159890 [14] WPIDS
DOC. NO. CPI: C2000-049883
TITLE: Construct useful for altering cellulose content in plants and increasing digestibility of plant material contains a DNA sequence encoding the E1 **cellulase** enzyme from **Acidothermus cellulolyticus**.
DERWENT CLASS: C06 D16 P13
INVENTOR(S): HIMMEL, M E; SCHAAF, D J; STALKER, D M; THOMAS, S R
PATENT ASSIGNEE(S): (CALJ) CALGENE LLC
COUNTRY COUNT: 24
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 6013860	A	20000111	(200014)*		10
WO 2000005381	A2	20000203	(200014)	EN	
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: BR CA JP MX US					
EP 1017824	A1	20000712	(200036)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
BR 9906651	A	20000829	(200046)		
JP 2002522023	W	20020723	(200263)		40
MX 2000002971	A1	20011201	(200282)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 6013860	A	US 1998-122533	19980724
WO 2000005381	A2	WO 1999-US16579	19990723
EP 1017824	A1	EP 1999-934174	19990723
		WO 1999-US16579	19990723
BR 9906651	A	BR 1999-6651	19990723
		WO 1999-US16579	19990723
JP 2002522023	W	WO 1999-US16579	19990723
		JP 2000-561327	19990723
MX 2000002971	A1	MX 2000-2971	20000324

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 1017824	A1	Based on	WO 200005381
BR 9906651	A	Based on	WO 200005381
JP 2002522023	W	Based on	WO 200005381

PRIORITY APPLN. INFO: US 1998-122533 19980724
AB US 6013860 A UPAB: 20000320
NOVELTY - Construct (I) comprising, in the 5' to 3' direction, a promoter

functional in a plant cell, a DNA sequence (**II**) encoding the E1 **cellulase** from **Acidothermus cellulolyticus** and a transcription terminator region, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(a) plant cell containing (I);

(b) plants, or their seeds or parts, containing cells of (a); and

(c) a method for altering cellulose content in plant tissue by introducing (I) into plant cells.

USE - (I) are used to produce plants having altered, specifically reduced cellulose content, resulting in material of improved digestibility. More generally, thermophilic **cellulases** are useful for biomass conversion (e.g. to sugars, for conversion to fuel alcohol), in textile finishing, production of detergent additives, in food and beverage processing, as feed additives, for silage production, and in fermentation, paper and pulp manufacture.

ADVANTAGE - When expressed in plants, (**II**) retains its thermophilic properties, i.e. increased activity at over 45 deg. C, so it will not be active under growing conditions but can be activated during post-harvest processing. A further safeguard against activation in plants is provided by sequestering (**II**) to a plastid where it is isolated from its substrate.

Dwg.0/3

L5 ANSWER 10 OF 19 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-087663 [08] WPIDS
DOC. NO. CPI: C2000-024514
TITLE: Isolated domains of **Acidothermus cellulolyticus** E1 **endoglucanase** useful for labeling or modifying a cellulose and for purifying or immobilizing a binding domain fusion protein to cellulose.
DERWENT CLASS: C06 D16
INVENTOR(S): ADNEY, W S; HIMMEL, M E; LAYMON, R A; THOMAS, S R
PATENT ASSIGNEE(S): (MIDE) MIDWEST RES INST
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 2226898	A1	19990925	(200008)*	EN	85

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
CA 2226898	A1	CA 1998-2226898	19980325

PRIORITY APPLN. INFO: CA 1998-2226898 19980325
AB CA 2226898 A UPAB: 20000215
NOVELTY - A compound comprising amino acid sequence (I) or a domain variant, consisting of residues 420-521 of the **Acidothermus cellulolyticus** E1 **endoglucanase** sequence (**II**) of 562 amino acids given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated and purified nucleic acid (**III**) encoding (I) or its domain variant;

(2) a hybrid protein (IV) comprising (I) or its domain variant;

(3) a nucleic acid encoding (IV);

(4) a chemical derivative (V) of (I);

(5) a chemical derivative (VI) of (IV);

(6) a method of modifying a polysaccharide surface comprising

contacting the polysaccharide with (I) or a hybrid protein comprising (I);

(7) a method of purifying (IV) comprising contacting the fusion protein with a polysaccharide matrix;

(8) a method of immobilizing (IV) on a support comprising contacting the hybrid protein with a polysaccharide matrix;

(9) a method of producing (I), domain variant of (I) or a hybrid protein comprising (I) comprising:

(a) inserting a nucleic acid sequence encoding (I), domain variant or hybrid protein into a vector;

(b) introducing the vector into a host cell; and

(c) expressing the nucleic acid of step (a); and

(10) a domain variant of (**III**) encoding a compound which comprises a defined sequence of 110 amino acids given in the specification.

USE - The **cellulose binding domain** of the E1 **endoglucanase** (E1 **CBD**) is useful in labeling or modifying a cellulose or other polysaccharide surface and in purifying or immobilizing a binding domain fusion protein to cellulose or other polysaccharide. The modified cellulose can then be used in textile, pulp, paper, chemical and pharmaceutical industries. A fusion protein comprising E1 **CBD** can be used to modify the chemical or physical properties of a cellulose or polysaccharide matrix column and to roughen or disrupt a cellulose or polysaccharide fiber.

E1 **endoglucanase** is a beta -1,4-**endoglucanase** or endocellulase

ADVANTAGE - The **cellulose binding domain** of the E1 **endoglucanase** (E1 **CBD**) exhibits greater stability at pH 4-8 and has an optimum temperature for stability of 83 deg. C which is not found in **CBD** from organisms not adapted to thermal temperatures.

Dwg.0/7

L5 ANSWER 11 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1999:144350 BIOSIS

DOCUMENT NUMBER: PREV199900144350

TITLE: Molecular diversity of thermophilic cellulolytic and hemicellulolytic bacteria.

AUTHOR(S): Bergquist, Peter L. (1); Gibbs, Moreland D.; Morris, Daniel D.; Te'o, V. S. Junior; Saul, David J.; Morgan, Hugh W.

CORPORATE SOURCE: (1) Sch. Biol. Sci., Macquarie Univ., Sydney, NSW 2109 Australia

SOURCE: FEMS Microbiology Ecology, (Feb., 1999) Vol. 28, No. 2, pp. 99-110.
ISSN: 0168-6496.

DOCUMENT TYPE: General Review

LANGUAGE: English

AB Many thermophilic bacteria belong to groups with deep phylogenetic lineages and ancestral forms were established before the occurrence of eucaryotes that produced cellulose and hemicellulose. Thus they may have acquired their beta-glycanase genes from more recent mesophilic bacteria. Most research has focussed on extremely thermophilic eubacteria growing above 65degreeC under anaerobic conditions. Only recently have aerobic cellulolytic thermophiles been described from widely separated lineages (for example, Rhodothermus marinus, Caldibacillus cellulovorans). Many thermophilic bacteria produce **cellulases** and xylanases that have novel structures, with additional protein domains not identified with their catalytic activity. Many of these enzymes are multifunctional and code for more than one catalytic activity. This type of enzyme structure was first identified in the extreme thermophile Caldicellulosiruptor saccharolyticus. There is a general relatedness evident between catalytic domains, **cellulose binding domains** and other ancillary domains, which suggests that there may have been significant lateral gene transfer in the evolution of these microorganisms. Detailed molecular studies show that there is variation in the sequences of these

related but not identical genes from taxonomically widely-separated organisms.

L5 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:76000 CAPLUS
DOCUMENT NUMBER: 128:151117
TITLE: Improved thermostability in **cellulase** by production of the C-terminal truncated catalytic domain
INVENTOR(S): Adney, William S.; Thomas, Steven R.; Baker, John O.; Himmel, Michael E.; Chou, Yat-Chen
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: U.S., 19 pp., Cont.-in-part of U.S. 5,536,655.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5712142	A	19980127	US 1996-604913	19960222
US 5110735	A	19920505	US 1989-412434	19890926
EP 885955	A2	19981223	EP 1998-108104	19900827
EP 885955	A3	19990407		
R: DE, FR, GB				
US 5275944	A	19940104	US 1992-826089	19920127
US 5366884	A	19941122	US 1993-125115	19930921
US 5536655	A	19960716	US 1994-276213	19940715

PRIORITY APPLN. INFO.:

US 1989-412434	19890926
US 1992-826089	19920127
US 1993-125115	19930921
US 1994-276213	19940715
EP 1990-914450	19900827

AB The gene encoding **Acidothermus cellulolyticus** E1 **endoglucanase** is cloned and expressed in Pichia pastoris. A new modified E1 **endoglucanase** enzyme comprising the catalytic domain (residues 1-358) of the full-size, mature E1 enzyme demonstrates enhanced thermostability and is produced by 2 methods. The first method of producing the new modified E1 is proteolytic cleavage to remove the **cellulose binding domain** and linker peptide of the full size E1. The second method of producing the new modified E1 is genetic truncation of the gene encoding the full size E1 so that the catalytic domain is expressed in the expression product.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:556109 CAPLUS
DOCUMENT NUMBER: 123:4072
TITLE: Quantitation of **Acidothermus cellulolyticus** E1 **endoglucanase** and Thermomonospora fusca E3 exoglucanase using enzyme-linked immunosorbent assay (ELISA)
AUTHOR(S): Nieves, Rafael A.; Chou, Yat-Chen; Himmel, Michael E.; Thomas, Steven R.
CORPORATE SOURCE: Appl. Biol. Sci. Branch, Natl. Renewable Energy Lab., Golden, CO, 80401, USA
SOURCE: Applied Biochemistry and Biotechnology (1995), 51/52, 211-23
CODEN: ABIBDL; ISSN: 0273-2289
PUBLISHER: Humana
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two distinct quant. indirect ELISAs were developed to det. the concn. of recombinant **cellulase** components in culture filtrates. A monoclonal antibody (E1P7) was used as the primary antibody in developing an ELISA specific for A. **cellulolyticus** E1 **cellulase** (**endoglucanase**) (I). Likewise, a polyclonal rabbit serum (Ab684) was used to develop an ELISA specific for Thermomonospora fusca E3 exocellobiohydrolase (exoglucanase) (**II**). Dose-response curves indicated a dynamic range for both assays between 0.01 and 0.08 .mu.g/mL (1-8 ng/assay) when purified enzymes were used as stds. These ELISAs were used to est. the concns. of secreted recombinant I and/or **II** in culture supernatants of Streptomyces lividans strain TK24 in which the corresponding genes were cloned and expressed.

L5 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 1996:11558 CAPLUS
DOCUMENT NUMBER: 124:49197
TITLE: Synergism between purified bacterial and fungal **cellulases**
AUTHOR(S): Baker, John O.; Adney, William S.; Thomas, Steven R.; Nieves, Rafael A.; Chou, Yat-Chen; Vinzant, Todd B.; Tucker, Melvin P.; Laymon, Robert A.; Himmel, Michael E.
CORPORATE SOURCE: Alternative Fuels Div., National Renewable Energy Laboratory, Golden, CO, 80401-3393, USA
SOURCE: ACS Symposium Series (1995), 618(Enzymatic Degradation of Insoluble Carbohydrates), 113-41
CODEN: ACSMC8; ISSN: 0097-6156
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A standardized comparative study measured glucose release and synergistic effects in the solubilization of microcryst. cellulose by binary mixts. of 11 fungal and bacterial **cellulases** (eight **endoglucanases** and three exoglucanases). Evaluation of 16 endo/exo pairs revealed that bacterial/fungal hybrid pairs are very effective in solubilizing microcryst. cellulose. Of nine bacterial/fungal hybrid pairs studied, six were ranked among the nine most synergistic combinations, and six bacterial/fungal pairs were also among the top nine pairs in terms of sol.-sugar release. One hybrid pair (**Acidothermus cellulolyticus** E1 and Trichoderma reesei CBH I) was ranked first in both synergism and sugar-release. In exo/exo synergism expts., the performance of Thermomonospora fusca E3 confirmed its mode of action as "CBH **II**-like" (i.e., E3 is synergistic with T. reesei CBH I but not with T. reesei CBH **II**). Studies of endo/endo interactions suggested a possible means of categorizing **endoglucanases** in terms of substrate specificity.

L5 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 1995:278405 CAPLUS
DOCUMENT NUMBER: 122:75491
TITLE: Thermostable **endoglucanase II** of **Acidothermus cellulolyticus** and method for its purification
INVENTOR(S): Adney, William S.; Thomas, Steven R.; Nieves, Rafael A.; Himmel, Michael E.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: U.S., 15 pp. Cont.-in-part of U.S. 5,275,944.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

US 5366884	A	19941122	US 1993-125115	19930921
US 5110735	A	19920505	US 1989-412434	19890926
EP 885955	A2	19981223	EP 1998-108104	19900827
EP 885955	A3	19990407		
R: DE, FR, GB				
US 5275944	A	19940104	US 1992-826089	19920127
US 5432075	A	19950711	US 1994-275995	19940715
US 5536655	A	19960716	US 1994-276213	19940715
US 5712142	A	19980127	US 1996-604913	19960222

PRIORITY APPLN. INFO.:

US 1989-412434	19890926
US 1992-826089	19920127
EP 1990-914450	19900827
US 1993-125115	19930921
US 1994-276213	19940715

AB A purified low mol. wt. **endoglucanase II** from **Acidothermus cellulolyticus** (ATCC 43068) is disclosed. The **endoglucanase** is water sol., possesses both C1, and Cx types of enzyme activity, a high degree of stability toward heat, and exhibits optimum temp. activity at about 81.degree. at pH's from about 2 to about 9, and at a inactivation temp. of about 100.degree. at pH's from about 2 to about 9. A process for prepg. the enzyme comprises concg. the A. **cellulolyticus** culture broth by using ammonium sulfate pptn. (between 40% and 60% satd. solns.) or by ultrafiltration using an Amicon ultrafiltration app. equipped with PM-10 membranes. The conc. can be stored at -20.degree. in the presence of 20% glycerol for periods greater than a year with no loss in enzyme activity. The high mol. wt. **endoglucanase** complex produced by this process has a temp. optimum near 65.degree.. **Endoglucanase II** (and **endoglucanase** I) are prepd. from the complex by high-performance size-exclusion and ion exchange column chromatog.

L5 ANSWER 16 OF 19 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 95:49759 SCISEARCH
THE GENUINE ARTICLE: BB85E
TITLE: **CELLULASE** PRODUCTION TECHNOLOGY - EVALUATION OF CURRENT STATUS
AUTHOR: PHILIPPIDIS G P (Reprint)
CORPORATE SOURCE: NATL RENEWABLE ENERGY LAB, BIOPROC BRANCH, 1617 COLE BLVD, GOLDEN, CO, 80401 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: ACS SYMPOSIUM SERIES, (1994) Vol. 566, pp. 188-217. ISSN: 0097-6156.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: ENGLISH
REFERENCE COUNT: 90

L5 ANSWER 17 OF 19 LIFESCI COPYRIGHT 2003 CSA on STN
ACCESSION NUMBER: 95:6787 LIFESCI
TITLE: Thermostable purified **endoglucanase II** from **Acidothermus cellulolyticus** ATCC
AUTHOR: Adney, W.S.; Thomas, S.R.; Nieves, R.A.; Himmel, M.E.
CORPORATE SOURCE: Midwest Research Inst., Kansas City, MO (USA)
SOURCE: (1994) . US Patent 5,366,884.
DOCUMENT TYPE: Patent
FILE SEGMENT: A
LANGUAGE: English
AB A purified low molecular weight **cellulase endoglucanase II** from **Acidothermus cellulolyticus** (ATCC 43068).

L5 ANSWER 18 OF 19 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1991-08371 BIOTECHDS
TITLE: Thermostable purified **cellulase** complex;

produced by **Acidothermus cellulolyticus**
for use in industrial cellulolysis

PATENT ASSIGNEE: Midwest-Res.Inst.
PATENT INFO: WO 9105039 18 Apr 1991
APPLICATION INFO: WO 1990-US4868 27 Aug 1990
PRIORITY INFO: US 1989-412434 26 Sep 1989
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1991-132847 [18]

AB The following are claimed: (a) a pure, high mol.wt. thermostable **cellulase** (EC-3.2.1.4) (I) of mol.wt. 156,600-203,400 from **Acidothermus cellulolyticus** ATCC 43068, which is water-soluble, has both C1 (**cellulase**) and Cx (cellobiohydrolase, EC-3.2.1.91) activities, has an optimum temp. of 65 deg at pH 2-9 and an inactivation temp. of about 90 deg at pH 2-9; and (b) a pure low mol.wt. thermostable **cellulase** (**II**) from ATCC 43068, which is water-soluble, has both C1 and Cx activities, an optimum temp. of about 80 deg at pH 2-9 and an inactivation temp. of about 110 deg at pH 2-9. (I) is prepared by cultivating the bacterium, filtering the crude **cellulase** complex by ultrafiltration, separating unpurified high mol.wt. fractions by size exclusion chromatography, and anion-exchange chromatography, eluting with a 0-25 mM NaCl gradient. (**II**) is produced by culturing the bacterium, separating the crude **cellulase** complex by ultrafiltration, separating high and low mol.wt. fractions by size exclusion chromatography, CsCl precipitation, and ionexchange chromatography of low mol.wt. fractions, eluting with a 0-50 mM NaCl gradient. (21pp)

L5 ANSWER 19 OF 19 CABA COPYRIGHT 2003 CABI on STN
ACCESSION NUMBER: 1998:11583 CABA
DOCUMENT NUMBER: 980300073
TITLE: Thermostable purified **endoglucanase II** from **Acidothermus cellulolyticus** ATCC 43068
AUTHOR: Adney, W. S.; Thomas, S. R.; Nieves, R. A.; Himmel, M. E.
CORPORATE SOURCE: Midwest Research Institute
PATENT INFORMATION: 19940000
SOURCE: United States Patent, No. US 5366884, pp. 15. A 21.09.93-US-125115, P 22.11.94. Continuation-in-part of US 5275944.
DOCUMENT TYPE: Patent
LANGUAGE: English

AB A purified low-MW **cellulase** called **endoglucanase II** from **Acidothermus cellulolyticus** (ATCC 43068) is disclosed for use in biomass saccharification. It is water-soluble, possesses both C1 and Cx types of enzyme activity, and is very stable towards heat. In the pH range 2-9, its optimum temperature for activity (CMC degradation) is approx equal to 81 deg C and its inactivation temperature is approx equal to 100 deg .